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Blank qPCR Master Mix (2x)

REAL TIME PCR KIT

Kit Components:

Blank qPCR Master Mix (2x)

Component	**Cat. No.** E0403-01 100 reactions, 25 µl each, 2.5 ml [1x] final volume	**Cat. No.** E0403-02 200 reactions, 25 µl each, 5 ml [1x] final volume	**Cat. No.** E0403-03 1000 reactions, 25 µl each, 25 ml [1x] final volume
Blank qPCR Master Mix (2x)	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml
UNG (uracil-N-glycosylase) 1 U/µl	30 µl	55 µl	270 µl
25 mM $MgCl_2$	0.2 ml	0.4 ml	2.0 ml
Water, nuclease free	1 x 1.25 ml	2 x 1.25 ml	10 x 1.25 ml

Storage:
Store at -20˚C in the dark for long-term storage or at 4˚C for up to 1 month.

Description:

- Blank qPCR Master Mix (2x) is a universal solution for quantitative real-time PCR and two-step real-time RT-PCR and is compatible with most real-time PCR cyclers available.
- Blank qPCR Master Mix is a HotStart DNA polymerase master mix with several qPCR specific optimizations. The mix does not contain any preadded dyes. Thus it is possible to set up qPCR assays without the need for tedious optimization of a basic qPCR master mix but with a maximum of flexibility regarding experimental design. The mix allows the experimenter to add custom qPCR dyes, to choose arbitrary channels for fluorometric detection as well as to adjust and hand-optimize dye concentrations.
- The master mix contains Perpetual *Taq* DNA Polymerase, optimized reaction buffer and dNTPs (dTTP is partially replaced with dUTP).
- Perpetual *Taq* DNA Polymerase contains a recombinant *Taq* DNA Polymerase bound to anti-*Taq* monoclonal antibodies that block polymerase activity at moderate temperatures.
- The polymerase activity is restored during the initial denaturation step, when amplification reactions are heated at 95˚C for at least two minutes.
- Use of the "hot start" enzyme prevents extension of misprimed products and primer-dimers during reaction setup leading to higher specificity and sensitivity of PCR reactions.
- The polymerase enables convenient reaction setup at room temperature.
- Blank qPCR Master Mix (2x) contains dUTP, which partially replaces dTTP. It allows the optional use of an uracil-N-glycosylase (UNG) to prevent carryover contamination between reactions. UNG removes uracil from any dU-containing contaminating amplicons, leaving abasic sites and making DNA molecules susceptible to hydrolysis during the initial denaturation step.
- There are two variants of the kit: without ROX and with ROX Solution provided separately. The use of ROX passive reference dye is necessary for all real-time PCR cyclers from Applied Biosystems and optional for cyclers from Stratagene. ROX compensates for variations of fluorescent signal between wells due to slight differences in reaction volume and fluorescence fluctuations. ROX is not involved in PCR reaction and does not interfere with real-time PCR on any instrument. Refer to the table below to determine the recommended amount of ROX (25 µM) required for a specific PCR cycler.

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REAL TIME PCR PROTOCOL

qPCR- Protocol

Recommended amounts of ROX for a specific real-time PCR cycler

Instrument	Amount of ROX per 25 µl reaction	Final ROX concentration
Applied Biosystems: 7300, 7900HT, StepOne, StepOnePlus, ABI PRISM 7000 and 7700	0.3-0.5 µl	300-500 nM
Applied Biosystems: 7500 Stratagene: Mx3000P, Mx3005P, Mx4000	0.3-0.5 µl 10 x diluted (in water)	30-50 nM
PCR machines from other manufacturers: Bio-Rad, Roche, Corbett, Eppendorf, Cepheid, etc.	Not required	-

Preparation of PCR Reaction:

Component	Volume/Reaction	Final Concentration
Blank qPCR Master Mix (2x)	12.5 µl	1 x 2.5 mM $MgCl_2$
Forward Primer	Variable	0.3-0.5 µM
Reverse Primer	Variable	0.3-0.5 µM
Template DNA	Variable	500 ng
qPCR dye of choice	Variable	Variable
Optional: $MgCl_2$ (25 mM)	0 - 1.5 µl	2.5 - 4 mM
Optional: ROX Solution, 25 µM	0.3-0.5 µl or 0.3-0.5 µl 10 x diluted	300-500 nM 30-50 nM
Optional: UNG (uracil-N-glycosylase) 1 U/µl	0.25 µl	0.25 U / reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. Minimize exposure of ROX to light during handling to avoid loss of fluorescent signal intensity.
2. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
3. The optimal amlicon length in real-time PCR is 70 - 200 bp.
4. Thaw, gently vortex and briefly centrifuge all solutions.
5. Set up PCR reactions at room temperature. Use of Blank qPCR Master Mix (2x) allows room temperature reaction setup.
6. Prepare a reaction master mix by adding all the reaction components except template DNA.
7. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
8. Add template DNA/cDNA (500 ng/reaction) to the individual PCR tubes or wells containing the reaction mix. For two-step RT-PCR, the volume of cDNA added should not exceed 10% of the final PCR volume.
9. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
10. Place the samples in the cycler and start the program.
11. The standard concentration of $MgCl_2$ in real-time PCR reactions is 2.5 mM (as provided with the 1 x Blank qPCR Master Mix). In most cases this concentration will produce optimal results. However, if a higher $MgCl_2$ concentration is required, prepare a 25 mM $MgCl_2$ stock solution (or request us to ship an aliquot along with your order) and add an appropriate amount to the reaction. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 25 µl will add 25 nmol $MgCl_2$ and thus increase total $MgCl_2$ reaction concentration in 1.0 mM.
12. A final primer concentration of 0.3 - 0.5 µM is usually optimal, but can be individually optimized in a range of 0.1 µM to 1 µM. The recommended starting concentration is 0.3 µM. Raising primer concentration may increase PCR efficiency, but negatively affects PCR specificity. The optimal primer concentration depends on the individual reaction and the real-time PCR cycler used.
13. Readjust the threshold value for analysis of every run.
14. When using a Bio-Rad iCycler iQ or MyiQ instruments collect well factors at the beginning of each experiment. Use an external well factor plate according to the manufacturer's recommendations. Well factors are used to compensate for any excitation or pipetting variations.

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REALTIME PCR PROTOCOL

qPCR- Protocol - Thermal Cycling Conditions

Thermal Cycling Conditions:

Step	Tempera-ture	Time	Number of Cycles
Optional: UNG pre-treatment	50˚C	2 min	1
Initial Denaturation	95˚C	10 min	1
Denaturation Annealing Extension Optional: Data acquisition	95˚C 50-60˚C 72˚C X˚C (see Note 5)	15 s 30 s 30 s 15 s	35-45
Cooling	4˚C	Indefinite	1

Notes:

1. An incubation step of 50˚C for 2 minutes must be added if a uracil-N-glycosylase is used to prevent carryover contamination. UNG degrades any dUMP-containing PCR products.
2. During the initial denaturation step UNG and antibodies that block *Taq* DNA Polymerase are inactivated. The anti-*Taq* antibodies and UNG require at least 2 min or 10 min incubation at 95˚C, respectively. When UNG is not used in PCR reaction the duration of the initial denaturation step can be reduced to 2-5 min at 95˚C.
3. UNG activity may be partially restored at temperatures lower than 55˚C due to refolding. It is recommended to perform PCR using a temperature equal 55˚C or above for the annealing step. Upon completion of PCR, cool reactions to 4˚C and load directly on a gel or store frozen.
4. Melting curve analysis should be performed to verify the specificity and identity of PCR products. Melting curve analysis is an analysis step built into the software of real-time cyclers. Melting curve data between 65˚C and 95˚C should be acquired.
5. Data acquisition should be performed during the extension step. To suppress fluorescence readings caused by the generation of primer-dimers, it is possible to add an additional data acquisition step to the protocol. Discrimination between fluorescence readings induced by PCR product formation from primer-dimer accumulation is possible, if the T_m of primer-dimers is lower than the T_m of specific PCR products (T_m are determined during melting curve analysis). For this reason, choose a suitable temperature for the data acquisition step well above the T_m of primer-dimers, but approximately 3˚C below the T_m of the specific product.
6. Always check the PCR product specificity by gel electrophoresis when designing a new assay. Melting temperatures of the specific product and primer-dimers may overlap.